Functional alterations in jejunal myenteric neurons during inflammation in nematode-infected guinea pigs

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Palmer, Jeffrey M., Margaret Wong-Riley, and Keith A. Sharkey. Functional alterations in jejunal myenteric neurons during inflammation in nematode-infected guinea pigs. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G922–G935, 1998.—Intracellular recordings of jejunal myenteric neurons with an afterspike hyperpolarization (AH) from Trichinella spiralis-infected animals showed enhanced excitability on days 3, 6, and 10 postinfection (PI) compared with uninfected animals. Lower membrane potential, increased membrane input resistance, decreased threshold for action potential discharge, decreased AH amplitude and duration, and increased fast excitatory postsynaptic potential amplitude and duration were characteristic of neuronal recordings from infected animals. Concurrent with electrophysiological changes during T. spiralis infection, increased cytochrome oxidase activity, a marker of neuronal metabolic activity, and the expression of nuclear c-Fos immunoreactivity, an indicator of transcriptional-translational activity, were also observed in myenteric ganglion cells. Double-labeling for calbindin-immunoreactive myenteric neurons revealed that ~50% of these neurons also expressed increased c-Fos immunoreactivity during T. spiralis infection. Myeloperoxidase activity was significantly higher in the junction of T. spiralis-infected guinea pigs on days 3, 6, and 10 PI vs. uninfected counterparts. The expression of c-Fos in calbindin-immunoreactive neurons together with enhanced neuronal electrical and metabolic activity during nematode-induced intestinal inflammation suggests the onset of excitation-transcription coupled changes in enteric neural microcircuits.

enteric nervous system; excitation-transcription coupling; cytochrome oxidase; c-Fos immunoreactivity; calbindin immunoreactivity; Trichinella spiralis

GASTROINTESTINAL inflammation caused by parasitic nematode infection evokes functional disturbances in enteric physiological effector systems, including the contractile activities of the smooth muscle layers and their regulation by intrinsic nerves (6, 10). Altered intestinal motility is a prominent feature of the integrated physiological response of the host animal to enteric nematode infection, as well as contributing to symptoms of host gastrointestinal distress and morbidity (6). Experimental enteric infections with the nematodes Nippostrongylus brasiliensis and Trichinella spiralis are now well known to induce disturbances in small intestinal motility in vivo that have been characterized by increased transit rates of nonabsorbable markers (7, 15) and altered smooth muscle myoelectric and contractile activities in parasitized animals (13, 36). Altered small bowel motility during experimental nematode infections is associated with significant functional alterations of the individual components (i.e., smooth muscle and intrinsic nerves) that comprise the intestinal neuromuscular apparatus (10, 11, 32).

Intestinal inflammation associated both with enteric nematode parasites and other causes leads to marked changes in the structure and neurochemical content of intestinal nerves (40). Thus alterations in intrinsic neuronal function in the intestine can be inferred and indeed have been shown to exist. For example, several lines of evidence have indicated that the release of endogenous stores of neurotransmitters from both extrinsic and intrinsic nerve endings is impaired during enteric nematode infection. Electrical field stimulation of intrinsic inhibitory nerves was significantly reduced in its ability to cause relaxation of jejunal circular smooth muscle from N. brasiliensis-infected rats (14). Collins and co-workers (11, 43) have shown that stimulated release of the endogenous radiolabeled neurotransmitters [3H]acetylcholine and [3H]norepinephrine from jejunal longitudinal muscle-myenteric plexus (LMP) preparations was significantly decreased, whereas stimulated release of substance P was significantly increased (42) in T. spiralis-infected rats compared with uninfected rats. The hypothesis that a functional reorganization of the intrinsic neural control of intestinal motility is triggered by mucosal inflammation in the nematode-parasitized mammalian intestine is supported in part by the observations described above. To date, however, investigation of functional alterations in single enteric neurons from the nematode-parasitized intestine has not been performed.

Evidence obtained within the last several years has confirmed the role of intrinsic neurons contained within the myenteric and submucosal plexuses of the enteric nervous system (ENS) in the normal control and coordination of local enteric sensorimotor reflexes evoked by luminal stimuli (3, 5, 29, 31, 38). In particular, intestinal primary afferent nerves have recently become a focal point for investigation because of their proposed role in host defense against various noxious stimuli (24). In addition to extrinsic primary afferent nerves, the ENS itself has primary afferent neurons located in both the submucosal (28) and myenteric plexuses (3, 31). In the myenteric plexus of the guinea pig small intestine, neurons acting as intrinsic primary afferent neurons and contained in self-reinforcing networks have been identified electrophysiologically as nerve cells having a significant afterspike hyperpolarization (AH-type neuron) and a multipolar Dogiel type II morphology (3–5, 17, 31, 41). The capacity of myenteric AH neurons to respond electrophysiologically with

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heightened excitability to changes in gut wall tension or tactile and chemical stimulation of the mucosa has demonstrated the afferent sensory-like role of this neuronal class in the ENS (3, 5, 18, 29, 31). AH neurons contained within local ganglionic networks and by virtue of their multipolar morphology have been shown to not only make synaptic contacts with each other, but they also can synaptically drive the activities of another class of second-order neurons, designated as S-type neurons with a lamellar unipolar morphology (Dogiel type I), that appear to function as interneurons and motor neurons in the ENS (17, 46). These findings strongly suggest a pivotal multirole capacity for the AH class of myenteric neuron in the guinea pig small bowel. The effects of mucosal inflammation on the properties and behavior of these intrinsic nerve cells and the circuits of which they are a part are presently unknown.

The aim of our work was to investigate concurrently the electrical, metabolic, and transcriptional activity of myenteric neurons in response to experimental enteric T. spiralis infection in the guinea pig. Changes in the electrical and synaptic behavior of myenteric AH neurons were selected as the focal points for our intracellular electrophysiological studies because of the inherent inexcitability of these neurons during basal recording conditions (7, 33, 46), the high frequency of impairment of this class of myenteric neuron in electrophysiological investigation (7, 33, 46), and because of their proposed roles as key driver or gating neurons and as primary afferent neurons in the ENS (3, 5, 29, 31, 38, 41, 46).

We also examined myenteric ganglia for indications of increased metabolic activity using cytochrome oxidase (CO) histochemistry. Enhanced neuronal CO staining has been shown to be tightly coupled with increased electrical activity of both central and peripheral neurons, including myenteric neurons (25, 27, 33). Finally, the transcriptional and translational activation of cellular immediate early gene products was detected immunohistochemically in myenteric ganglion cells. Cellular immediate early genes c-fos and junB are sensitive markers whose increased expressions have been correlated with enhanced neuronal activation in the ENS (28, 37, 38). Our results suggest that mucosal inflammation during enteric nematode infection significantly affects the cellular neurophysiology of myenteric neurons manifested as upregulated electrical, metabolic, and transcriptional activation. The onset of excitation-transcription coupled changes might act as a signal for long-term modulation of neuronal activity within intrinsic reflex microcircuits.

**MATERIALS AND METHODS**

Animals and induction of inflammation. All procedures involving the use of live animals for these studies were reviewed and approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin (Milwaukee, WI), where these studies were initiated, and by the Animal Research Committee of Creighton University (Omaha, NE), where they were completed. Outbred, male, Hartley strain guinea pigs, *Cavia porcellus* (Harland Sprague Dawley, Indianapolis, IN), weighing 300–500 g served as host animals and were each orally inoculated with 8–10 × 10³ *T. spiralis* muscle-stage larvae that had been isolated using a previously described pepsin-hydrochloric acid digestion technique (19), and were administered oropharyngally via feeding tube in a 0.2 ml 0.85% saline vehicle bolus. Other guinea pigs served as age-matched uninfected controls and received only 0.2 ml of oropharyngally administered saline without the worms. All animals were fed a standard guinea pig chow diet supplemented with vitamin C and had free access to water during the course of the investigation.

Experimental protocol and design. LMMP preparations were obtained from uninfected control guinea pigs and *T. spiralis*-infected guinea pigs on days 3, 6, and 10 postinfection (PI). Segments of the proximal jejunum 5 cm distal to the ligament of Trietz and 20 cm in length were removed from adult guinea pigs, which had been killed by a stunning blow to the head and exsanguinated through a deep, sharp ventral incision across the neck. Exposure of the myenteric plexus was achieved with use of a previously described microdissection method (46) to prepare whole mounts of the longitudinal muscle layer and adherent myenteric plexus ganglia (LMMP) that were relatively free of overlying circular muscle. Jejunal LMMP preparations were then utilized for either intracellular electrophysiological experiments, CO histochemistry, or c-Fos immunohistochemistry. For CO histochemistry and c-Fos immunohistochemistry, LMMP tissues from both uninfected guinea pigs and *T. spiralis*-infected counterparts were processed concurrently on a given day PI to ensure similar incubation and reaction conditions.

Electrophysiological and pharmacological methods. The LMMP preparation was pinned to the Sylgard-coated bottom of a small-volume (1.5 ml) tissue chamber and perfused with a circulating Krebs solution warmed to 37°C and gassed with a mixture of 95% O₂-5% CO₂ (pH 7.3–7.4), at a rate of 8–10 ml/min. The composition of the Krebs solution (in mM) was 120 NaCl, 5.0 KCl, 1.2 MgCl₂, 1.35 NaH₂PO₄, 14.4 NaHCO₃, 2.54 CaCl₂, and 12.7 glucose. Myenteric ganglia were visualized with an inverted microscope (Diaphot, Nikon, St. Louis, MO) equipped with Hoffman modulation contrast optics and epi-illumination. Individual ganglia were immobilized between a pair of electrolytically tapered L-shaped tru-chrome orthodontic steel wires (100 µm length; Rocky Mountain Orthodontic, Denver, CO) that were positioned parallel to the long axis of the ganglion and perpendicular to the axis of the longitudinal muscle layer (46).

Transmembrane potentials of myenteric ganglion cells were recorded intracellularly with sharp glass micropipettes fabricated with a Brown-Flaming electrode puller (model P-87, Sutter Instruments, Novato, CA) and filled with 3 M KCl with direct current (DC) resistances ranging from 50 to 80 MΩ. The microelectrode was coupled to an electronic amplifier (Intra 767, World Precision Instruments, Sarasota, FL), which was equipped with a bridge circuit for simultaneous injection of electrical currents into the cell while recording the transmembrane electrotonic responses of the neuron. After impalement and stabilization of resting membrane potential, myenteric neurons were classified according to their active and passive electrical membrane properties. Electrical behavior, synthetically evoked responses, and responses to pharmacological agents were recorded digitally on videotape (Vetter Digital model 3000A PCM recorder, Vetter, Redersburg, PA) for later playback and computerized analysis using a MacLab 4-s data acquisition and analysis system (AD Instruments, Milford, MA) and a Macintosh Power PC 7100/66 computer (Apple Computer, Cupertino, CA).
Neuronal resting membrane potentials were determined from the amplifier electrometer once the voltage tracing displayed on the oscilloscope had stabilized and remained unchanged for at least 10 min. After a stable resting potential was obtained, injection of graded amplitude and duration square-wave current pulses from an electronic stimulator (model S48 stimulator, Astro-Med-Grass Instruments, Quincy, MA) through the recording microelectrode was initiated to evoke depolarized or hyperpolarized electrotonic membrane potentials to characterize the passive and active membrane properties of the neuron. The membrane input resistance of each neuron was calculated from the slope near the origin of the best-fit line determined from computerized plots of the current-voltage relationship for a given neuron during specific test conditions. Current injection was controlled from a step-pulse generator (Nihon Koden model SET-1201, Medical Systems, Greenvale, NY) to deliver a sequence of six, 200-ms duration hyperpolarizing square pulses with incremental increases of current at 1-s intervals. This sequence of pulses was repeated three times at 10- to 20-s intervals for control periods, during perfusion of applied neuroactive pharmacological agents, and during washout of these specific test agents from the tissue chamber.

Synaptic potentials were evoked by application of focal electrical shocks of varying frequency (0.1–0.5 Hz), duration (300–600 µs), and intensity (6–14 V) to neuronal processes coursing through interganglionic fiber tracts connecting the ganglion containing the impaled neuron with neighboring myenteric ganglia. Extracellular shocks applied to interganglionic connectives were delivered from the tips of Teflon-coated platinum-iridium wires (20 µm diam; Medwire, Mt. Vernon, NY) connected to an electronic stimulator (model S48 stimulator, Astro-Med-Grass Instruments).

Test agents for pharmacological studies of neuronal responses were delivered to the tissue chamber by addition of the substance at the desired concentration(s) to the perfusing Krebs solution. Alternatively, pharmacological agents were also delivered from fine glass pipettes positioned directly over the ganglion in which the neuron had been impaled that were connected to a picospritzer device (General Valve, Fairfield, NJ) capable of ejecting defined amounts of test agents from the pipettes with milliseconds pulses of pressurized nitrogen gas. Chemical agents used for these studies included ACh, TTX, hexamethonium, and atropine, all obtained from Sigma Chemical (St. Louis, MO).

CO histochemistry. Preparations of LMMP from uninfected control guinea pigs and from T. spiralis-infected counterparts were concurrently processed histochemically for neuronal CO activity (21). LMMP preparations were incubated for ~20 h in cold (4°C) 2% (wt/vol) saponin without agitation to permeabilize the myenteric ganglia. After saponin treatment, LMMP tissues were incubated in a reaction medium consisting of 0.05% (wt/vol) 3,3'-diaminobenzidine tetrahydrochloride and 0.03% (wt/vol) cytochrome C type III dissolved in 0.1 M sodium phosphate buffer containing 4% (wt/vol) sucrose at 37°C for 2 h with agitation. The reaction was stopped by flushing the tissues with three successive changes of cold (4°C) 0.1 M phosphate buffer. Tissues were then mounted on chrome-alum gelatin-coated slides, dehydrated, and coverslipped.

Intensities of CO reaction product were analyzed by optical densitometric measurements which were made with a Zeiss microscope fitted with a Zeiss PI-2 photometer and illuminated with a tungsten lamp. Comparisons were only made between the whole mount preparations of paired LMMP from uninfected and T. spiralis-infected guinea pigs that had been concurrently incubated and treated with the same reaction medium for the same lengths of time on the same day. Intensities were quantified in terms of the number of neurons reaching the CO labeling threshold per 25 ganglia, as well as the intensity of light transmission through neurons containing the reaction product. Densities of CO reaction product were determined within single-labeled neurons using a ×40 objective lens and a microdensitometry system as described previously (21). The optical density of the reaction product in each cell (the inverse of light transmission) provided a measure of the relative level of CO activity present. Optical density values were expressed in terms of arbitrary units, and they were then compiled and analyzed statistically.

Immunohistochemistry. Segments of proximal jejunum (4–5 cm in length) from groups of uninfected control guinea pigs and T. spiralis-infected guinea pigs were immunohistochemically stained for the expression of c-Fos as previously described (37). The intestine was cut open longitudinally along the mesenteric border and then pinned mucosa side up in cold (4°C) Krebs solution containing 10 µM nifedipine onto the bottom of a Sylgard-coated petri dish. This resulted in a full-thickness rectangular sheet of intestine which was gently stretched to a point at which firm resistance was encountered to further stretch. Tissues were fixed overnight (16–18 h) in modified Zamboni's fixative, permeabilized by washing in dimethylsulfoxide (3 changes for 10 min each), and subsequently washed in PBS (pH 7.4). Tissues were then further microdissected, removing the mucosa-submucosa layers and the overlying circular muscle to yield a LMMP preparation. Single- and double-labeling methods were utilized with a monoclonal antibody directed against the protooncogene protein product, c-Fos (antibody no. T161, generously provided by Dr. K. Riabowol, University of Calgary) alone or together with an anti-calbindin polyclonal antibody (Swant). Calbindin immunoreactivity has previously been shown to be present in all of the AH-type neurons with Dogiel type II morphology in the guinea pig small bowel (17, 18, 41). Tissues were incubated in primary antibodies for 48 h at 4°C. After washing in PBS (3 changes for 10 min each) tissue preparations were incubated for 1 h at 22°C with the fluorescent secondary antisera that consisted of goat anti-rabbit antibody conjugated to fluorescein isothiocyanate, and sheep antimouse antibody conjugated to CY3 (Sigma Chemical). After further washing tissues were mounted in bicarbonate-buffered glycerol (pH 8.6) and viewed with a Zeiss axioplan fluorescence microscope. The c-Fos-labeled nerve cells were counted in at least five myenteric ganglia per animal on days 3 (n = 2 animals) and 4 (n = 4) PI. Similarly, the number of c-Fos-labeled nerve cells that were also immunoreactive for calbindin were identified and counted.

MPO assay. Myeloperoxidase (MPO) activity was measured in extracts of full-thickness sections (200–300 mg) of guinea pig jejunum as an index of the intensity of inflammation due to T. spiralis-induced injury using a previously described spectrophotometric method (19). MPO levels were measured from gut tissues obtained from the same animals in which electrophysiological, CO, and c-Fos activities were investigated, providing us with a direct assessment of inflammation status and intensity with any detectable neuronal functional alterations due to nematode infection. Changes in optical density per minute were measured spectrophotometrically at 475 nm in triplicate, and data were recorded and analyzed with a computerized data acquisition and analysis system (MacLab, AD Instruments). Results were determined from a standard curve and expressed as units per milligram of wet tissue. A unit of MPO activity was defined as one micromole of H2O2 degraded per minute at 25°C.
Statistical analysis. All data are reported as means ± SE, and the number of animals studied is indicated in parentheses. Student's t-test and single-factor ANOVA were used to determine statistically significant differences among data obtained from uninfected control and T. spiralis-infected guinea pigs at various time points PI. Dunnett's post hoc multiple comparisons test was used to identify significantly different means after ANOVA. Computerized optical densitometric measurements of differences in the intensities of CO metric measurements of differences in the intensities of CO activity of individual myenteric neurons from LMMP tissues from uninfected and T. spiralis-infected guinea pigs were evaluated statistically by Student's t-test. For all statistical analyses a probability level of P < 0.05 was considered significant.

RESULTS

Neuronal electrical and synaptic behavior during jejunal T. spiralis infection. Intracellular electrophysiological recordings were obtained from T. spiralis-infected guinea pigs on days 3, 6, and 10 PI. Overall, a total of 201 neurons in jejunal LMMP preparations from 52 T. spiralis-infected guinea pigs were examined at these time points PI and compared with 64 neurons from 31 uninfected control animals. Ganglion cells were classified electrophysiologically according to previously accepted and published criteria for myenteric neurons from guinea pig small bowel. Four different types of neuronal electrical behavior were observed, including neurons that were classified as S, AH, type 3, and type 4 (see Refs. 4 and 46 for description of different properties and categories). All four types were encountered in ganglia from uninfected control guinea pigs, whereas only S, AH, and type 4 were recorded from ganglia of T. spiralis-infected animals. The proportions (percent of total neurons studied) of the different electrophysiological types of myenteric neurons studied in T. spiralis-infected vs. uninfected animals were 5, 14.9 vs. 12.5% AH, 75.6 vs. 71.8%; type 3, 0 vs. 1.5%; and type 4, 9.9 vs. 14%.

Our investigation was focused on the effects of T. spiralis-induced inflammation on the AH class of myenteric neurons. Recordings from these neurons during basal conditions are characterized by relatively high resting membrane potentials, low membrane input resistances, a TTX-insensitive action potential with a shoulder on the descending phase, and a prominent myenteric neuron type (S neurons) possesses a high basal activity level characterized by stimulus-evoked repetitive action potential discharge, spontaneous action potential discharge, and high membrane input resistances that could make it difficult to determine neuronal behavioral changes due to parasitic nematode-evoked inflammation.

Forty-six of the 64 neurons (71.8%) recorded from uninfected guinea pigs were AH neurons. In comparison, 152 of 201 cells (75.6%) recorded from T. spiralis-infected guinea pigs were of this same neuronal classification. Resting membrane potentials of AH neurons from T. spiralis-infected guinea pigs were significantly more depolarized on each of the days that were examined PI than those recorded from age-matched uninfected animals (Table 1). Membrane input resistances determined from the slopes of current-voltage plots were also significantly increased in AH neurons from T. spiralis-infected animals as early as day 3 PI (Table 1) and remained elevated through day 10 PI compared with AH neurons from uninfected animals. This finding indicated that membrane conductance was markedly decreased in these neurons during T. spiralis infection. Membrane input resistances were measured at least three times during the recording period to determine stability for a given neuron. In functional studies of myenteric AH neurons from normal animals, membrane depolarization accompanied by increased input resistance usually occurs only in response to excitatory agonist receptor-activated K+ channel closing (46) or Cl− channel opening (2). Together, these results suggested that an enhanced excitability state existed for

Table 1. Electrical and synaptic properties of AH-type myenteric neurons in guinea pig jejenum during Trichinella spiralis infection

<table>
<thead>
<tr>
<th>Property</th>
<th>Uninfected</th>
<th>Day 3 PI</th>
<th>Day 6 PI</th>
<th>Day 10 PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accommodation at threshold, no. of AP per current pulse</td>
<td>2.4 ± 1.3 (46)</td>
<td>8.3 ± 4.9* (34)</td>
<td>15.6 ± 4.2† (77)</td>
<td>9.5 ± 3.4‡ (41)</td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>65.3 ± 5.6 (46)</td>
<td>51.1 ± 4.3† (34)</td>
<td>47.1 ± 6.1‡ (77)</td>
<td>56.2 ± 5.7 (41)</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>101.1 ± 13.1 (46)</td>
<td>212.0 ± 38.9† (34)</td>
<td>254.0 ± 35.9‡ (77)</td>
<td>211.0 ± 66.1 (41)</td>
</tr>
<tr>
<td>Spontaneous AP discharge incidence, % of neurons</td>
<td>6.5% (3 of 46)</td>
<td>52.9% (18 of 34)</td>
<td>79% (60 of 77)</td>
<td>51% (21 of 41)</td>
</tr>
<tr>
<td>Anodal-break discharge incidence, % of neurons</td>
<td>10.9% (5 of 46)</td>
<td>73.5% (25 of 34)</td>
<td>84.4% (65 of 77)</td>
<td>43% (17 of 41)</td>
</tr>
<tr>
<td>Fast EPSP amplitude, mV</td>
<td>6.9 ± 1.6 (12)</td>
<td>13.7 ± 2.2† (21)</td>
<td>14.8 ± 2.4‡ (41)</td>
<td>8.9 ± 2.1 (19)</td>
</tr>
<tr>
<td>Fast EPSP duration, ms</td>
<td>18.4 ± 3.3 (12)</td>
<td>26.5 ± 4.1† (21)</td>
<td>28.7 ± 3.8‡ (41)</td>
<td>22.4 ± 4.7 (19)</td>
</tr>
</tbody>
</table>

Values are means ± SE except where indicated as a percentage. No. of neurons studied in parentheses: AH, afterhyperpolarization; PI, postinfection; AP, action potential; EPSP, excitatory postsynaptic potential. Statistically significant difference compared with uninfected control: *P < 0.05, †P < 0.01, ‡P < 0.001.
AH myenteric neurons during the inflammatory response due to *T. spiralis* infection.

Further confirmation of this finding was obtained when a significant increase in the number of action potentials was evoked by intrasomal injection of square-wave depolarizing current pulses of comparable amplitudes in AH neurons from *T. spiralis*-infected animals compared with uninfected controls (Table 1). In neurons from uninfected guinea pigs (Fig. 1, A and B), it was difficult to evoke action potential discharge, often necessitating intrasomal injection of increasingly larger depolarizing current pulses through the recording microelectrode to evoke one to three action potentials at the highest current amount passed. In contrast, AH neurons from *T. spiralis*-infected guinea pigs (Fig. 1, C and D) showed a remarkable lack of accommodation at threshold (repetitive discharge), and an increase in the amplitude of the depolarization increased the number of evoked action potentials further. This response of AH neurons in *T. spiralis*-parasitized guinea pigs was a consistent finding throughout the enteric phase of infection and occurred at much lower current strengths compared with uninfected animals. This finding is evidence for a lowered threshold for excitation in AH myenteric neurons from *T. spiralis*-inflamed LMMP.

The occurrence of anodal-break excitation (action potential discharge) at the offset of intrasomal injection of square-wave hyperpolarizing current pulses was another sign of the increased excitability in myenteric AH neurons of *T. spiralis*-infected guinea pigs. The incidence of anodal-break excitation in recordings of AH neurons from *T. spiralis*-infected animals increased through the duration of the enteric phase of infection peaking by day 6 PI (Table 1). Electrotonic potentials evoked by injection of increasingly larger steps of hyperpolarizing current through the microelectrode into a neuron from an uninfected guinea pig are shown in Fig. 1B. In comparison, Fig. 1D shows the same current injection protocol utilized in a recording from an AH neuron from a *T. spiralis*-infected guinea pig on day 6 PI that resulted in hyperpolarizing electrotonic potential changes of larger amplitude evoked at lower current strengths than neurons from uninfected controls.

Alterations in the waveform of the action potential as well as the amplitudes and durations of the AH were also observed in recordings of myenteric AH neurons during enteric *T. spiralis* infection (Table 2; Fig. 2). Comparative analysis of action potential waveforms showed a small but significant decrease in action potential duration as early as day 3 PI and persisting through day 10 PI compared with those from uninfected animals (Fig. 2, A and B). However, maximum action potential amplitudes were not significantly different between uninfected and *T. spiralis*-parasitized guinea pigs throughout the course of infection. The rate of change of voltage vs. time of the action potential downstroke (calculated as the average slope) of AH neurons from *T. spiralis*-infected animals showed a more rapid rate of descent compared with those from uninfected animals at all time points examined PI (Table 2). These data suggest that the inward Ca²⁺ current that occurs during the action potential and

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**Fig. 1.** Electrical excitability of myenteric afterspike hyperpolarization (AH) neurons recorded from *Trichinella spiralis*-infected guinea pigs on day 6 postinfection (PI). A: electrotonic potentials and single action potential discharge evoked by intrasomal injection of increasingly larger steps of depolarizing current through the recording electrode in a neuron from an uninfected guinea pig. B: electrotonic potentials evoked by intrasomal injection of increasingly larger steps of hyperpolarizing current in a neuron from an uninfected guinea pig. C: repetitive action potential discharge evoked by intrasomal injection of a single step of depolarizing current into a neuron from a *T. spiralis*-infected guinea pig. D: anodal-break excitation (i.e., action potential discharge at offset of stimulus) evoked by intrasomal injection of increasingly larger steps of hyperpolarizing current into a neuron from a *T. spiralis*-infected guinea pig. All injected square-wave current pulses were 200-ms duration and 0.2–0.8 nA magnitude.
which is represented by the $\text{Ca}^{2+}$ shoulder of the downstroke is significantly reduced in AH neurons from ganglia in T. spiralis-infected tissues or that other channels responsible for outward rectification are blocked.

The peak amplitudes and durations of the AH after stimulus-evoked action potential discharge were significantly decreased as well in AH neurons from myenteric ganglia in T. spiralis-infected animals compared with uninfected animals (Table 2; Fig. 2, C and D). This was determined using the same duration of injected current (20 ms) but variable current strengths based on the threshold potential of the neuron so that only a single action potential would be discharged. Summated AHs were also evoked with use of a 200-ms depolarizing current pulse, and in a few AH neurons from uninfected guinea pigs a maximum of three action potentials could be evoked but only at large current strengths reflective of the relatively low excitability state of these neurons under normal conditions (Fig. 2C). In marked contrast, a summated AH could be evoked in nearly all AH neurons recorded from ganglia in T. spiralis-parasitized animals but not uninfected controls (Fig. 3, A and B). This observation was additional confirmation that somal excitability of these neurons had been dramatically increased during nema-
Enhanced electrical excitability of myenteric AH neurons in T. spiralis-infected jejunal LMMP was also evident in the high proportion of spontaneously active neurons (unstimulated action potential discharge) that were observed in our recordings at every time point examined PI compared with uninfected controls (Table 1; Fig. 4, A–C). This high level of spontaneous excitability does not occur in normally quiescent AH neurons. Spontaneous action potential discharge in these neurons was TTX-insensitive (Fig. 4B). Furthermore, spontaneous activity did not appear to be synaptically driven because exposure to 0.3 µM TTX, a neurotoxin that blocks axonal action potential conduction and neurotransmitter release, only slightly attenuated the discharge rate but did not abolish unstimulated firing of action potentials (Fig. 4B). With use of intrasomal injection of constant hyperpolarizing DC current, spontaneous action potential discharge was completely abolished at a current-clamped membrane potential around −85 mV (data not shown).

Twelve of 46 AH neurons in uninfected guinea pigs (26%) and 81 of 152 AH neurons from T. spiralis-infected guinea pigs (53%) had fast excitatory postsynaptic potentials (EPSP). The ability of myenteric AH neurons to produce suprathreshold fast EPSP after synaptic activation was also enhanced in ganglia obtained from T. spiralis-infected guinea pigs on days 3–10 PI compared with uninfected controls. Data presented in Table 1 show that significant increases were detected in both the amplitude and duration of fast EPSP evoked in AH neurons from T. spiralis-inflected LMMP. Fast EPSP were evoked in AH neurons using graded-strength focal electrical shocks applied extracellularly to interganglionic fiber tracts (Fig. 5, A and B) and were hexamethonium sensitive (200 µM). Application of ACh microejected from a fine-tipped pipette onto the impaled neuron in the presence of hexamethonium still produced corresponding increases in number of action potentials discharged together with a summation in the amplitude and duration of the AH in an AH neuron recorded from a T. spiralis-infected guinea pig on day 6 PI (filled dots beneath each event represent the number of spikes discharged in a one-to-one relationship with the number of shocks applied; horizontal bar, 4.8 s; vertical bar, 10 mV).

Neuronal CO activity during jejunal T. spiralis infection. Histochemical staining of LMMP whole mounts for detection of changes in neuronal CO activity demonstrated a significant increase in staining intensity due to enteric T. spiralis infection on days 3, 6, and 10 PI. Photomicrographs of myenteric ganglia revealed not only an increased staining intensity of individual ganglion cells in tissues from T. spiralis-infected guinea pigs compared with uninfected counterparts (Fig. 6, A and B) but also an increase in the number of neurons with high CO activity. These findings were widespread throughout the myenteric plexuses of T. spiralis-infected vs. uninfected animals (data not shown).

Measurements of the optical densities of 50 individual ganglion cells selected in a blinded and randomized fashion from each whole mount preparation were used to quantify CO reactivity changes due to T. spiralis infection. Comparisons were only made between preparations that had been concurrently incu-
bated and stained over the same 24-h period from a pair of age-matched uninfected and T. spiralis-infected guinea pigs examined on a specific day PI. Significant increases in the number of myenteric ganglion cells with higher reaction product densities and hence higher optical density readings occurred in T. spiralis-infected animals on each day examined PI compared with uninfected counterparts (i.e., for each time point, no. of uninfected animals, \( n = 3 \); no. of infected animals, \( n = 3 \)). These results were reproduced in tissues obtained.

Fig. 4. Spontaneous action potential discharge in AH myenteric neurons recorded in jejunal ganglia from T. spiralis-infected guinea pigs on day 6 PI. A: spontaneous action potential discharge and action potential waveform before addition of TTX to Krebs buffer solution superfusing longitudinal muscle-myenteric plexus (LMMP) preparation in recording chamber. B: TTX-insensitive spontaneous action potential discharge and waveform after a 10-min exposure to TTX (0.3 µM). C: spontaneous action potential discharge and waveform after 10-min washout of TTX from recording chamber. Arrowheads, effect of TTX on Na\(^+\)-dependent component of action potential upstroke.

Fig. 5. Enhanced fast excitatory synaptic transmission in myenteric AH neurons recorded from T. spiralis-infected guinea pigs on day 6 PI. A: fast excitatory postsynaptic potentials (EPSP) recorded at current clamped membrane potential of \(-59\) mV. Extracellular stimulus pulse parameters: 600 µs duration, 0.1 Hz frequency, and increasing stimulus strength (numbered arrows) as shown at 8.2 (1), 8.7 (2), and 9.2 V (3). B: fast EPSP recorded at current clamped membrane potential of \(-60\) mV. Extracellular stimulus pulse parameters: 500 µs duration, 0.1 Hz frequency, and increasing stimulus strength (numbered arrows) as shown at 6.4 (1), 6.8 (2), and 7.0 (3) V.
Fig. 6. Cytochrome oxidase (CO) histochemical reactivities of jejunal myenteric ganglion cells in whole mount longitudinal muscle-myenteric plexus (LMMP) preparations from uninfected (A) and T. spiralis-infected (B) age-matched guinea pigs on day 6 PI (magnification ×100). Note markedly increased CO reaction product densities of compared ganglion cells (indicated by filled arrowheads) in LMMP whole mount from T. spiralis-infected guinea pig vs. uninfected counterpart.

and densitometrically analyzed from three different sets of uninfected and T. spiralis-infected animals. Figure 7 shows results expressed in terms of arbitrary optical density units obtained from paired uninfected-infected LMMP tissues examined on day 3 (Fig. 7A, uninfected 0.666 ± 0.013 vs. infected 0.812 ± 0.0147; P < 0.0001), day 6 (Fig. 7B, uninfected 0.756 ± 0.0104 vs. infected 1.022 ± 0.0194; P < 0.0001), and day 10 (Fig. 7C, uninfected 0.734 ± 0.012 vs. infected 0.968 ± 0.0147; P < 0.0001) PI. These data indicate that overall metabolic activity in myenteric neurons is significantly elevated during the mucosal inflammatory response to T. spiralis. The findings of increased CO reactivity in myenteric ganglion cells from T. spiralis-infected guinea pigs correlated with parasite-induced neuronal electrophysiological changes and elevations in jejunal tissue MPO activity (see below). These results are consistent with the coupling of enhanced electrical excitability to increased neuronal metabolic activity (21, 22, 27, 33) and serve as corroborative evidence that excitability of AH neurons is enhanced during T. spiralis-induced inflammation.

Neuronal nuclear protooncogene expression during jejunal T. spiralis infection. Changes in the expression of neuronal immediate early gene products in myenteric ganglia were examined using standard fluorescence microscopy of LMMP whole mounts immunostained with specific antibodies directed against c-Fos on days 1, 3, 4, and 6 PI. In control animals, no c-Fos immunoreactivity was detected in the myenteric plexus at any time after inoculation with saline vehicle alone (Fig. 8A). The c-Fos immunoreactivity was detected on days 3–6 PI in neuronal nuclei of the myenteric plexus from parasitized guinea pigs (Fig. 8, A and B) but not at the other time points studied. The quality of this staining differed slightly from that seen in other circumstances. In myenteric ganglia from T. spiralis-infected animals, c-Fos immunoreactivity had a slightly punctate appearance, whereas normally it tends to have a homogenous smooth appearance in the nucleus (with an unstained nucleolus). The reason for the change in character of the staining is unclear at this time. An immediate early gene response in myenteric neurons appears likely to occur during the enteric phase of T. spiralis infection in association with the early and peak stages of jejunal mucosal injury and inflammation that attends epithelial invasion and occupation by the worms. The marked increase in c-Fos immunoreactivity observed in myenteric plexus preparations from infected animals on days 3–6 PI was coincident with enhanced neuronal electrical excitability, metabolic activity, and peak levels of tissue MPO activity (see below) during jejunal T. spiralis infection in guinea pigs. Quantitative estimates of the peak c-Fos immunoreactivity at days 3 and 4 revealed that ~50% of the ganglion cells were c-Fos positive (45 ± 5 cells/ganglion, n = 28 ganglia from six animals, assuming about 100 neurons/ganglion) (24, 35).

Double-labeling studies for calbindin immunoreactivity (Fig. 8C), a marker of AH cells in the myenteric plexus of the guinea pig, revealed that 52 ± 7% of the calbindin immunoreactive neurons expressed c-Fos, whereas 12 ± 2% of the c-Fos nerve cells were calbindin positive. Thus these findings suggest that a significant proportion (~50%) of those intrinsic neurons that could be potentially considered to be primary afferent neurons due to their calbindin immunoreactivity (17, 18, 41) expressed an increased transcriptional and translational activity. We should stress that it is also apparent from our results that the increase in c-Fos immunoreactivity was a widespread phenomenon that most likely included other classes of myenteric plexus neurons, possibly motor neurons and interneurons. Thus these data are comparable to the widespread increase we observed in CO staining intensity of neurons in the myenteric plexus of the T. spiralis-inflected jejunum that also more than likely included other types of neurons.

MPO activity during T. spiralis-induced jejunal inflammation. Determination of tissue MPO activity as a measure of granulocyte infiltration is a standard mea-
sure of acute inflammatory reactions in the mucosa or in full-thickness specimens of gastrointestinal tissue (19), and correlates with the intensity of the inflammatory response. The activity of MPO in extracted tissues from the jejunum of guinea pigs during the enteric phase of T. spiralis infection was significantly elevated as early as day 3 PI (P < 0.05), peaked by day 6 PI (P < 0.001), and was still increased on day 10 PI (P < 0.01) compared with jejunal tissues from uninfected guinea pigs (Fig. 9). A maximum increase of ∼10-fold occurred in the mean MPO activity in tissues from T. spiralis-infected guinea pigs on day 6 PI compared with the mean MPO activity detected in jejunal tissues from uninfected control animals.

DISCUSSION

Our results demonstrate that the electrical and synaptic behavior of myenteric AH neurons is upregulated toward an increased state of excitability during invasion of the jejunal mucosa by enteric stages of the parasitic nematode T. spiralis. Functional excitability of jejunal AH neurons recorded from T. spiralis-infected guinea pigs on days 3, 6, and 10 PI was shown by lower mean resting membrane potentials, increased membrane input resistances, decreased duration of action potentials, and decreased amplitude and duration of postspike hyperpolarizing afterpotentials in these nerve cells compared with those recorded from uninfected animals. Enhanced excitability was also evidenced by an increased incidence of spontaneous action potential discharge and anodal break excitation in AH-type neurons. Additionally, when focal stimulation was applied to interganglionic fiber tracts entering the ganglion in which the impaled AH neuron was located, we detected increased amplitude and duration of evoked fast EPSP that attained threshold for action potential discharge. There was also a marked increase in axonal activation of antidromic action potential invasion of AH neurons observed in infected animals.

In the present investigation, the increased excitability observed in AH neurons from nematode parasitized jejunum was temporally associated with both histopathological changes (data not shown) and significant elevation of MPO activity in extracted jejunal tissues. These findings were consistent with previous work using this particular animal model for enteric host-parasite interactions (1, 8) and verified the inflamed state of the T. spiralis-infected small bowel. Quantification of MPO activity is a reliable index of inflammation intensity in mucosal, submucosal, and smooth muscle tissues during T. spiralis infections (19, 32). MPO activity was used as an indicator of the influx of increased numbers of granulocytic myeloid inflammatory cells into the mucosa-submucosa and deeper intestinal tissue that release proinflammatory mediators. The response to T. spiralis infection in our study occurred as early as day 3 PI, with a peak in MPO activity detected on day 6 PI. Increased MPO levels on days 3, 6, and 10 PI with T. spiralis are well correlated temporally with the occurrence of functional disturbances in intestinal motility and secretion in vivo and
in vitro (1, 6, 7, 13, 19, 36), altered contractile behavior of isolated smooth muscle strips in vitro (32), and altered release of neurotransmitters from the myenteric plexus in vitro (11, 42, 43) in rat and guinea pig hosts.

We corroborated our electrophysiological results with a histochemical method designed to gauge the presence of increased metabolic activation of neurons in jejunal myenteric ganglia from *T. spiralis*-infected guinea pigs. CO has been shown to be a sensitive and reliable marker for increased activity in central and peripheral neurons (25, 27, 33). Colchicine, cholera toxin, and the neurotoxins, 6-hydroxydopamine, 5,7-dihydroxytryptamine, and veratridine have all been shown to increase the intensity of CO reactivity in enteric neurons (27, 33). Mawe and Gershon (33) successfully used this technique to demonstrate that a functional heterogeneity in the state of neuronal activation exists in myenteric plexus ganglia of the guinea pig small bowel under normal conditions and that veratridine-stimulated increases in the intensity (i.e., optical density) of CO reaction product in myenteric ganglion cells correlated closely with increased electrical excitability of myenteric AH neurons. Thus enhanced electrical excitability in AH neurons, as well as in other central and peripheral neurons (25, 27, 33), is tightly coupled to a corresponding increase in aerobic metabolism. Our results are consistent with a correlation between increased electrical and metabolic activation of AH neurons during enteric *T. spiralis* infection. The largest increase in CO intensity occurred on day 6 PI and coincided with the peak increase detected in MPO activity of jejunal tissues from infected animals. Our findings also suggest that during the inflammatory process there is a generalized increase in neuronal activation within the plexus indicative of a decreased functional heterogeneity in the jejunal myenteric ganglia. Wong-Riley and co-workers have demonstrated that stimulated increases in neuronal CO activity are dependent primarily on changes induced in the regulation of the amount of enzyme protein present in the cell.

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**Fig. 8.** c-Fos immunoreactivity (IR) in LMMP whole mounts from jejunal myenteric plexus from uninfected (A) and *T. spiralis*-infected guinea pig (B). Calbindin IR shown in C is from same preparation as shown in B on day 3 PI. c-Fos IR was absent from myenteric plexus in control animals (A). After infection, nuclear neuronal c-Fos IR was found in about 45–50% of myenteric neurons (B). Double-labeling with antibodies raised against calbindin revealed extensive overlap (arrows) between c-Fos and calbindin IR in myenteric neurons. Scale bar, 50 µm.

**Fig. 9.** Myeloperoxidase (MPO) activity (means ± SE) in jejunal tissue measured as index of inflammation intensity evoked by enteric infection in guinea pigs with *T. spiralis* on days 3, 6, and 10 PI compared with uninfected controls (number of animals from which tissues were obtained for MPO assay is indicated at base of each bar). *P < 0.05. **P < 0.01. ***P < 0.001.
that transcriptional activation of both nuclear and mitochondrial subunit genes for CO is regulated by the level of neuronal electrical activity (22).

Based on the results presented above, we can speculate that the functional consequences of upregulated excitability of AH neurons during T. spiralis infection in guinea pig jejunum could involve altered somal gating properties of these multipolar and potentially multifunctional nerve cells. This electrophysiological class of neuron is a key component of both the sensory and motor limbs of local reflex arcs in the intestine (3, 5, 29, 31). This class of neuron has been proposed to regulate and facilitate the buildup and spread of excitation throughout the myenteric plexus based on stimulus-evoked modulation of the inherent inexcitability of the neuronal soma (46). Sustained membrane depolarization of AH neurons, similar to that occurring during the slow EPSP and dependent on K⁺ channel closure (30, 46), permits incoming excitation from the synaptically activated neurites or soma to propagate to outgoing neurites that target other neurons or drive effector tissue activity. The lower resting membrane potentials we observed in AH neurons from T. spiralis-infected jejunum would seem to assure that threshold for action potential discharge will be attained and propagated and could increase synaptic effectiveness and amplification vital for coordination of effector tissues responding to the noxious presence of the worms in the mucosal epithelium.

Another key finding of our work consisted of significant decreases observed in the amplitude and duration of AHs in AH neurons from T. spiralis-infected LMMP. This finding clearly demonstrated that a key mechanism governing excitability of these nerve cells, involving a receptor-operated Ca²⁺-activated K⁺ conductance that acts to limit action potential discharge frequency (30, 46), was altered during enteric T. spiralis infection. This interconversion of the functional state of AH neurons from one of high action potential accommodation (i.e., low excitability) to low accommodation (i.e., high excitability) of action potential discharge resulted in increased firing frequency of stimulus-evoked spikes.

Our data are consistent with that presented by Kunze and colleagues (31) who showed that under conditions of variable stretch AH neurons in the myenteric plexus of the guinea pig ileum are also able to convert from a low state to a high state of excitability. This functional property of AH neurons in the ENS is reminiscent of the intrinsic integrative properties of mammalian central and peripheral autonomic neurons and invertebrate sensory neurons that adapt their rate of spike discharge in response to the tone or strength of a conditioning stimulus that has altered the postspike hyperpolarizing afterpotential in these cells (12, 39, 45). For example, modulatory changes in the hyperpolarizing afterpotential are known to occur as a result of long-term potentiation in mammalian hippocampal neurons (12) and in conditioned learning and training in neurons of the marine invertebrate Aplysia (23), which reflect changes in neuronal sensory plasticity. Walters and co-workers have characterized inflammation-induced changes in the electrical and synaptic behavior of Aplysia sensory neurons in response to axonal injury (20, 45) and periaxonal inflammation (9) that are nearly identical to our results in mammalian myenteric neurons. Inflammation-induced changes in neuronal properties in Aplysia included lower resting membrane potential, decreased threshold for action potential discharge, decreased amplitude of AH, decreased accommodation of stimulus-evoked spike discharge, increased membrane input resistance, and increased amplitude and duration of evoked fast EPSP. We also observed an enhancement of fast excitatory synaptic transmission in LMMP from T. spiralis-infused guinea pigs. This finding suggests an upregulated sensitivity of the postsynaptic neuronal membrane to neurotransmitters and other neuroactive factors released during nematode-induced inflammation that prime the cellular regulatory mechanisms involved in promoting neuronal responsiveness to excitatory stimuli.

It is possible that the alterations we observed in the electrophysiology of myenteric AH neurons might reflect, in whole or in part, the effects of the nematode-induced intestinal inflammation. Neuronal damage, injury, or stress could be due to the release and presence of reactive oxygen metabolites (19), the formation of nitric oxide-dependent nitrating species like peroxynitrite (34), and other proinflammatory and anti-parasitic factors that are known to be released by the increased numbers of inflammatory and immune effector cells recruited to the nematode-infected small bowel (6, 16). However, other findings obtained utilizing physiological approaches suggest that this is not the only possibility that could account for the spectrum of functional alterations manifested by the nematode-parasitized intestine. For example, evidence presented by Alizadeh et al. (1) clearly demonstrated the occurrence of highly organized and coordinated net aboral propulsive movements of isolated jejunal segments from T. spiralis-infected guinea pigs on days 10–20 PI, whereas segments from uninfected counterparts did not demonstrate such activity. Isolated gut segments capable of generating propulsive complexes in vitro would require an intact, viable intrinsic nervous system. Furthermore, nematode-induced inflammation has been shown to include the production and release of factors such as histamine, serotonin, neuropeptides, prostanooids, and other arachidonic acid-derived mediators, all of which have been shown to have significant neuroactive properties (6, 46) and would not necessarily exert deleterious effects on neuronal function. Thus, it seems reasonable to infer that such highly organized propulsive events present only in the T. spiralis-infected jejunum are unlikely to be mediated by damaged intrinsic nerve cells whose function would be compromised. Instead, the observations of Alizadeh et al. (1) together with our present data strongly implicate an adaptive, plastic response leading to an increased level of intrinsic neurally activated intestinal motor organization and coordination during nematode infection and inflammation.
Electrical and metabolic activation of AH myenteric neurons persisted throughout the enteric phase of T. spiralis infection in the guinea pig jejunum. Marked changes in the parameters of excitability we examined, but most significantly in the postspike hyperpolarizing afterpotential which is an important regulatory event, led us to investigate the potential basis for these long-lasting changes in neuronal activity. Events such as the AH are known to be modulated by stimuli acting via receptor-induced intracellular second messengers that ultimately activate protein phosphorylation and changes in gene expression (46). It is now well established that excitation-transcription coupling in spinal sensory neurons induces changes in nociceptive processing that can produce significant functional changes for action potential activation (35). Protein products of immediate-early genes such as c-fos are involved in cellular stimulus-transcription coupling effectively acting as “third” messengers regulating transcription of target genes that affect neuronal phenotypic expression in response to extracellular stimuli (35, 44). Traub et al. (44) have reported a large induction of Fos-like immunoreactivity in rat lumbosacral spinal cord neurons after noxious colorectal distension. Other models of nerve injury and stimulation have also been shown to result in the differential induction of Fos and Jun protein expression (35, 38, 40). Our results showing induction of c-Fos immunoreactivity as early as day 3 PI are consistent with the notion that excitation-transcription coupling also occurs in myenteric neurons during T. spiralis-induced inflammation. Nearly one-half of the total neurons in the myenteric plexus showed nuclear Fos immunoreactivity in infected animals. Because the typical neuronal induction and expression of c-fos immunoreactivity usually has a rapid onset (i.e., within 60 min of stimulation) and a relatively short duration (i.e., 2–4 h) (35, 38, 40, 44), the persistent increased immunoreactivity may be due to changes other than electrical excitability. When calbindin immunoreactivity was also examined in these same tissues we found that slightly more than 50% of the c-Fos immunoreactive neurons were also calbindin immunoreactive. Because neuronal calbindin immunoreactivity in the guinea pig myenteric plexus has been shown to be a marker of AH-type neurons that can act as primary afferent neurons (17, 18, 41), our data suggest the possibility that a significant number of those AH neurons serving in a multifunctional capacity as primary afferent neurons within the myenteric plexus of T. spiralis-infamed tissues could have become activated during infection.

Our data are different from those of Ritter et al. (38) who were unable to demonstrate increased expression of c-Fos in calbindin immunoreactive neurons stimulated by distension, peristalsis, and forskolin application. The reasons for this discrepancy are unclear but might be reflective of the nature of the stimuli, possibly the actions of released inflammatory mediators in our T. spiralis-infected animals that affect the excitability of the AH-calbindin immunoreactive neurons. We believe these results strongly support our hypothesis that a functional reorganization of intrinsic neural function is initiated during the enteric phase of T. spiralis infection involving transcriptional and translational activation of neuronal immediate-early genes.

In conclusion, our intracellular electrophysiological investigations have revealed the occurrence of functional alterations in single enteric neurons during intestinal inflammation induced by enteric parasitism. Our results clearly demonstrate that the electrical and metabolic activities of jejunal myenteric AH neurons are significantly increased during nematode infection. These changes are associated with markedly elevated levels of the enzyme MPO, an index of granulocyte infiltration into gut tissues. Furthermore, we have presented evidence of the occurrence of excitation-transcription coupling in a significant proportion of myenteric plexus neurons during T. spiralis infection, as well as in a large percentage of those cells that are calbindin immunoreactive and possibly intrinsic primary afferent neurons. Collectively, these findings suggest that a functional reorganization of intrinsic neural function is initiated during small intestinal nematode infection that could be involved in the activation of intestinal motor responses that may subserve adaptive or host defense purposes.

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